

iGEM TU/e 2016

Biomedical Engineering

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1 Ligation

Estimated bench time: 20 minutes

Estimated total time: 19 hours

Purpose: Ligating the digested vector and the digested insert(s) so that a circular plasmid is created.

It is essential to work with gloves at all times to protect the DNA from DNase activity.

1.1 Materials

- 10x T4 ligase buffer
- Autoclaved H₂O
- Autoclaved PCR tubes
- Bucket with ice
- Digested insert
- Digested vector
- Pipettes and tips
- T4 ligase
- Thermal cycler

1.2 Setup & Protocol

- Construct a PCR mixture in the following way. Start with the component with the largest volume and end with T4 ligase. Keep the T4 ligase on ice.

Component	Quantity/mass/final concentration	Volume (uL)
Gel extracted digested vector (ng/uL)	100 ng	
PCR purified digested insert (ng/uL)	ng (vector:insert molar ratio 1:10)	
10x T4 ligase buffer	1x	5
T4 ligase		1
H ₂ O		
		50

- Mix well by pipetting up and down.
- Run the following PCR program:

Step	Temp (°C)	Time (min)
Incubation	16	1080 (18 hours)
Heat inactivation	65	10
Cooling	4	hold